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Variation, levels and profiles of organochlorines and brominated flame retardants in great tit (*Parus major*) eggs from different types of sampling locations in Flanders (Belgium)

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Abstract

Small-scale geographical variation in the occurrence of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) was investigated using the eggs of a terrestrial residential songbird species, the great tit (*Parus major*). In addition, we investigated the influence of the type of sampling location on the presence of these pollutants. To achieve this, 10 different sampling locations in Flanders (Belgium) were classified into 3 groups based on the extent of urbanisation, industrialisation and agriculture. The higher variance among sampling locations for the levels and profiles of PCBs and OCPs, suggests that local contamination sources are more important for the PCBs and OCPs compared to the PBDEs. Levels of PCBs and PBDEs were significantly higher in the industrialised sampling locations compared to the other locations. Sum PCB and sum PBDE levels reached up to 6050 and 79 ng/g lipid weight, respectively. PCBs and PBDEs were highly positively correlated for all groups, suggesting similar exposure pathways and/or mechanisms of accumulation. Significantly higher levels of OCPs (sum OCPs up to 2683 ng/g lipid weight) were detected in the rural sampling locations situated in a residential area. This suggests that local historical usage of OCPs by inhabitants may be an important source of contamination in Flanders. Contamination profiles differed also among the sampling locations. The rural sampling locations had a higher contribution of lower brominated BDE congeners, whereas the industrialised locations had a higher contribution of higher brominated congeners. The differences in contamination profiles among the sampling locations are probably due to differences in exposure. In conclusion, our results showed that the characteristics of a sampling location influence both the levels and profiles of PCBs, OCPs and PBDEs.

Keywords: Polychlorinated biphenyls; Organochlorine pesticides; Polybrominated diphenyl ethers; Flanders; Belgium; Industrialised; Rural; Great tit; Eggs

1. Introduction

The investigation of the occurrence of organohalogenated pollutants (OHPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), in the environment is of great importance because of their persistent character, bioaccumulative potential and adverse effects on both humans and wildlife (Vos et al., 2000). Due to the regulatory controls on the use of these compounds, there seems to be a decreasing temporal trend of PCBs and OCPs in biota (Jones and De Voogt, 1999). Nevertheless, concentrations in the environment are still high and exert a potential health risk. Recently, the presence of polybrominated diphenyl ethers (PBDEs) in the environment has received much attention (Law et al., 2006). PBDEs are a group of chemicals that are widely used in different materials because of their flame retarding properties. Large-scale production and use have led to their ubiquity in the environment and in biota, in which PBDE levels have increased rapidly (Hites, 2004). The association between high concentrations of some congeners and toxic effects in man and in wildlife (Darnerud, 2003), together with their persistent and bioaccumulative character, is of increasing

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concern and shows the need for more data on environmental levels of PBDEs.

Different animal species, including birds, are being used as sentinels of environmental pollution and as sentinels of human exposure (van der Schalie et al., 1999). Because practical and ethical reasons impede the sacrifice of free-living birds, methods for non-destructive biomonitoring of OHPs using eggs (Van den Steen et al., 2006), blood (Henriksen et al., 1998) or feathers (Jaspers et al., 2006, 2007; Van den Steen et al., 2007a) are highly favourable. Bird eggs have been successfully used to monitor OHPs in numerous studies (Donaldson et al., 1999; Elliott et al., 2005; Jaspers et al., 2005; Norstrom et al., 2002; Van den Steen et al., 2006) as females can pass contaminants stored in their body tissues into their eggs. Eggs can be easily collected and the collection of a single egg from a clutch is expected to have a minor effect on the population level (Furness, 1993). Because eggs can be readily sampled from the same location each year, long-term monitoring studies using eggs are also feasible. Moreover, the study of widespread bird species enables monitoring on a larger geographical scale. Although only a limited number of studies have investigated geographical variation of OHPs, such studies would be very valuable to obtain more information about the local usage and emission of OHPs. Passerine bird species are in particular useful to monitor local contamination with OHPs. In contrast with predatory species, residues in eggs of residential passerine species are expected to reflect local contamination much better because of their small home ranges, territories and foraging areas (Moore, 1966; Dauwe et al., 2006).

This study aimed to quantify the small-scale geographical variation in the occurrence of different OHPs (PCBs, PBDEs and OCPs) using the eggs of a terrestrial residential songbird species, the great tit (*Parus major*). In addition, we investigated the influence of the type of sampling location on the presence of OHPs. To achieve our objectives, we analysed great tit eggs from 10 different sampling locations in Flanders, a densely populated, heavily urbanised region in the northern part of Belgium (Europe) with a lot of industrial and agricultural activities. Locations were selected and classified in three groups based on the extent of urbanisation, industrialisation and agriculture. We predicted that OHPs would be highest in the rural sampling locations, while PCBs and PBDEs were expected to be linked to urbanisation and industrialisation (Jaward et al., 2004). A higher variance among sampling locations was expected for the levels and profiles of PCBs and OCPs, compared to the PBDEs. In contrast to point sources of PCB and OCP pollution, PBDEs are more widespread and enter the environment from more diverse sources (Siddiqi et al., 2003). Contamination profiles were examined to gather information about local exposure and contamination sources (Morrissey et al., 2004).

2. Materials and methods

The great tit is a small and resident insectivorous songbird that can be found throughout Europe and Northern Africa. It is one of the most common bird species in Europe and can be found in almost any wooded area. Because great tits are hole-nesting species that use nest boxes as roosting and nesting site and that are relatively easy to catch and study, they are perhaps the most intensively studied free-living bird species and they are increasingly being used in field ecotoxicological research (Dauwe et al., 2007; Eens et al., 1999; Hoff et al., 2005; Janssens et al., 2003).

During the breeding season of 2006, several volunteers collected great tit eggs from 10 different sampling locations in Flanders (Fig. 1 and Table 1). A previous study showed that variability was much lower within than among clutches and that laying order had no effect on PCBs, PBDEs and OCPs levels (Van den Steen et al., 2006). Therefore, one random egg per clutch was collected. Sampling locations were classified into three groups: 1) Group I: industrialised locations close to Antwerp harbour (Burchtse Weel, Ekeren, Vlietbos and Zandvliet). Antwerp harbour is the second largest harbour in Europe, one of the ten largest harbours worldwide and also the most productive (Antwerp Port Authority, 2006). A high concentration of chemical and petrochemical industry surrounds Antwerp harbour., 2) Group R1: rural locations situated in a less populated area (Kalmthout, Meeuwen–Gruitrode and Zoersel), 3) Group R2: rural locations situated in a residential area (Baal, Ham and Tessenderlo).

After collection, the eggs were weighed, measured and stored at −20 °C until sample preparation. Egg volume was calculated from the formula $V=0.51LB^2$ (Hoyt, 1979), where L is egg length and B is maximum egg breadth. A homogenised sample of approximately 0.5 g whole egg was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (ε-HCH, CB 46 and 143, BDE 77 and 128). Although not tested in the present samples, previous analyses

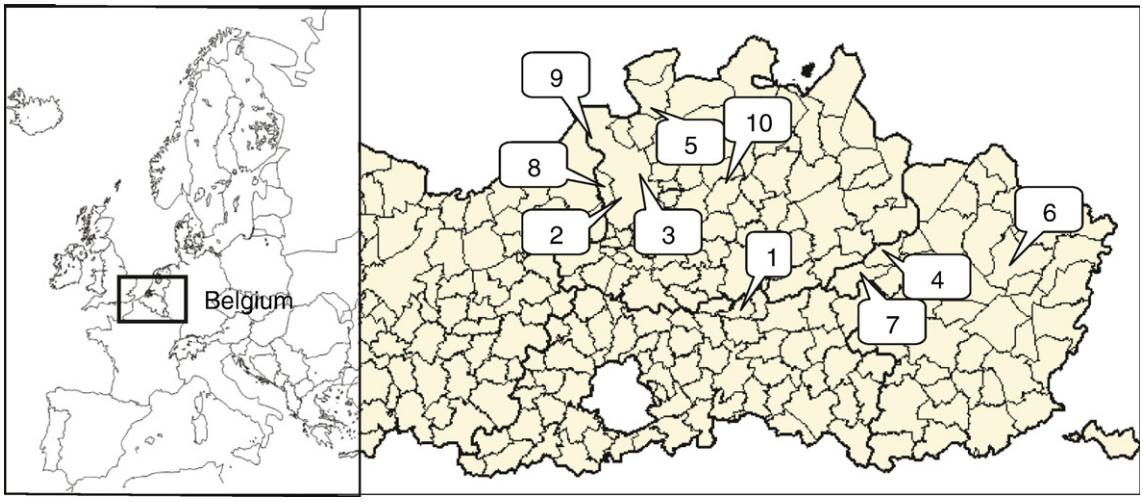

Fig. 1. Geographical distribution of the sampling locations in Flanders (Belgium): 1) Baal, 2) Burchtse Weel, 3) Ekeren, 4) Ham, 5) Kalmthout, 6) Meeuwen–Gruitrode, 7) Tessenderlo, 8) Vlietbos, 9) Zandvliet, 10) Zoersel.

Table 1
Description of sampling locations and number of eggs analysed per sampling location

Location	Group	Description	Number of eggs analysed
Burchtse Weel (Antwerp)	I	Recreation area bounded by the Scheldt, a residential area and industry.	7
Ekeren (Antwerp)	I	Recreation area (Muisbroek) surrounded by harbour industry, residential area and a busy motorway.	7
Vlietbos (Zwijndrecht)	I	Nature reserve close to busy motorways and industry	7
Zandvliet	I	Rural area with agriculture close to some important chemical harbour companies	7
Kalmthout	R1	Rural area with agriculture next to the nature reserve 'Kalmthoutse heide'	6
Meeuwen–Gruitrode	R1	Rural area with agriculture	6
Zoersel	R1	Rural area with agriculture	7
Baal (Tremelo)	R2	Residential area surrounded by agriculture	5
Ham	R2	Rural area with agriculture and industry	6
Tessenderlo	R2	Rural area with agriculture and industry	7

Sampling locations were classified into three groups: 1) Group I: industrialized locations close to Antwerp harbour (Burchtse Weel, Ekeren, Vlietbos and Zandvliet), 2) Group R1: rural locations situated in a thinly populated area (Kalmthout, Meeuwen–Gruitrode and Zoersel), 3) Group R2: rural locations situated in a residential area (Baal, Ham and Tessenderlo).

have shown that BDE 77 and BDE 128, used as internal standards, were not present in related samples from Belgium (Jaspers et al., 2006, Van den Steen et al., 2006). Further sample treatment and analysis were performed according to previously described methods (Jaspers et al., 2005; Van den Steen et al., 2006). Briefly, extraction was carried out with 100 ml hexane/acetone (3:1, *v/v*) in an automat Soxhlet extractor (Büchi, Flawil, Switzerland) in hot extraction mode for 2 h. The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned up on a column filled with ~8 g acidified silica and eluted with 15 ml hexane and 10 ml dichloromethane. The eluate was concentrated to 100 µl under a gentle nitrogen stream and transferred to an injection vial. In all samples, concentrations of 22 PCB congeners (CB 28, 31, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 163, 170, 180, 183, 187, 194, 196 and 199), 7 PBDE congeners (BDE 47, 49, 99, 100, 153, 154 and 183), dichlorodiphenyltrichloroethane (*p,p*'-and *o,p*'-DDT) and metabolites (*p,p*'-DDE and *p,p*'-DDD), hexachlorocyclohexanes (HCHs; α-, β- and γ-HCHs), chlordanes (CHLs; *cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN) and oxychlordane (OxC)), and hexachlorobenzene (HCB) were determined.

For the PCB analysis, an Agilent 6890 gas chromatograph (GC) connected with an Agilent 5973 mass spectrometer (MS) operated in electron ionisation (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the selected ion-monitoring (SIM) mode with two ions monitored for each PCB homologue group. One µl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min), pressure pulse 25 psi, pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (held for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, held for 12 min.

For the analysis of the OCPs and PBDEs, an Agilent 6890 GC connected with an Agilent 5973 MS operated in electron capture negative ionisation (ECNI) mode was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). Methane was used as moderating gas and the ion source, quadrupole and interface temperatures were set at 160, 150 and 300 °C, respectively. The MS was used in the SIM mode with two ions monitored for each pesticide in specific windows, while ions $m/z = 79$ and 81 were monitored for PBDEs during the entire run. One µl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 720 °C/min), pressure pulse 30 psi, pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.50 min, then increased to 220 °C at a rate of 15 °C/min (held for 2.0 min), further increased to 242 °C at a rate of 3 °C/min and finally raised to 300 °C at a rate of 40 °C/min, held for 15 min.

Multi-level calibration curves in the linear response interval of the detector were created for the quantification, and good correlation ($r^2 > 0.999$) was achieved. The identification of OHPs was based on the relative retention times (RRTs) to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values obtained for calibration standards was considered acceptable. The quality control was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs and OCPs in whale blubber), which has also indicative values for PBDEs, was used to test the method accuracy. Obtained values were deviating with less than 10% from the certified values. The quality control scheme is also assessed through regular participation to interlaboratory comparison exercises organized by AMAP and NIST. For each analyte, the mean procedural blank value was used for subtraction. BDE 47 and 99 had blank levels which were lower than 5% of the values found in the samples. Nevertheless, the blank levels were subtracted from the sample values. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs for the analysed compounds ranged between 0.1 and 3.5 ng/g lipid weight (lw).

Statistical calculations were performed using SPPS 14.0 for Windows on lipid-normalized concentrations. The level of significance was set at $\alpha = 0.05$ throughout this study. Before data analysis, samples with levels below LOQ were assigned a value of 1/2 * LOQ. Contaminants were excluded from the analysis if more than 50% of the samples had levels below LOQ. The sum PCB concentration of one sample in Kalmthout (14,000 ng/g lw) was higher than the value obtained from the formula $Q_3 + 1.5 * IQR$, where Q_3 is the third quartile and IQR the interquartile range, and therefore considered as an outlier. Subsequently, this sample was excluded from the data analysis. We performed nested ANCOVAs to investigate if there was a difference in contamination levels among the 3 considered groups and to check if there was a correlation between the contamination levels (sum PCBs, sum OCPs and sum PBDEs), egg sizes, weight and volume. Egg parameters were excluded from the model, because no significant effects were found. Post hoc tests were performed when there were significant effects. Correlations among the different OHPs were calculated using Pearson's correlations. Differences in variance of the contaminants among the groups were investigated using *F*-tests. The profiles of OCPs, PCBs and PBDEs were compared among the sampling locations by principal component analysis (PCA) on the percentage of the concentrations. Principal components with eigenvalues above 1 were considered to account for a significant contribution to the total variance according to the latent root criterion (Hair et al., 1998). Factor loadings and factor scores were determined and used in interpreting principal components patterns. Compounds with factor loadings greater than 0.65 on any PC were considered significant.

3. Results and discussion

3.1. Contaminant levels

PCBs constituted the major OHPs in the great tit eggs, with sum PCB concentrations ranging from 1082 ± 148 ng/g lw in Baal (Group R2) to 6050 ± 1084 ng/g lw in Ekeren (Group I; Fig. 2a). Sum PCB concentrations were significantly higher in the industrialised sampling locations compared to the rural locations (Nested ANOVA:

$F_{2,54}=17.79$, $p<0.001$; Tukey HSD: Group I>Group R1: $p<0.001$, Group I>Group R2: $p<0.001$; Fig. 2a). The higher concentrations of PCBs in group I are probably due to the proximity of Antwerp harbour. Several studies have reported high levels of PCBs in marine species and sediments close to harbours (de Boer et al., 2001; Barakat et al., 2002; Sprovieri et al., 2006; Voorspoels et al., 2004a). Previous studies with great tit eggs from sampling locations near Antwerp (Flanders, Belgium) showed concentrations similar to these of group I (Dauwe et al., 2006; Van den Steen et al., 2006). PCB concentrations in our study were in general higher compared to the concentrations in great tit eggs from Germany (Winter and Streit, 1992). On the other hand, compared to other studies using passerine eggs, PCB concentrations in our study were rather low (Arenal et al., 2004; Echols et al., 2004). The sampling locations in the latter studies were, however, highly polluted by past industrial activities and known for PCB contamination. In the present study, no specific sources of PCB contamination were known for the sampling locations. In addition, differences in feeding ecology may also contribute to differences in accumulation within and among species (Bustnes et al., 2000; Neigh et al., 2005).

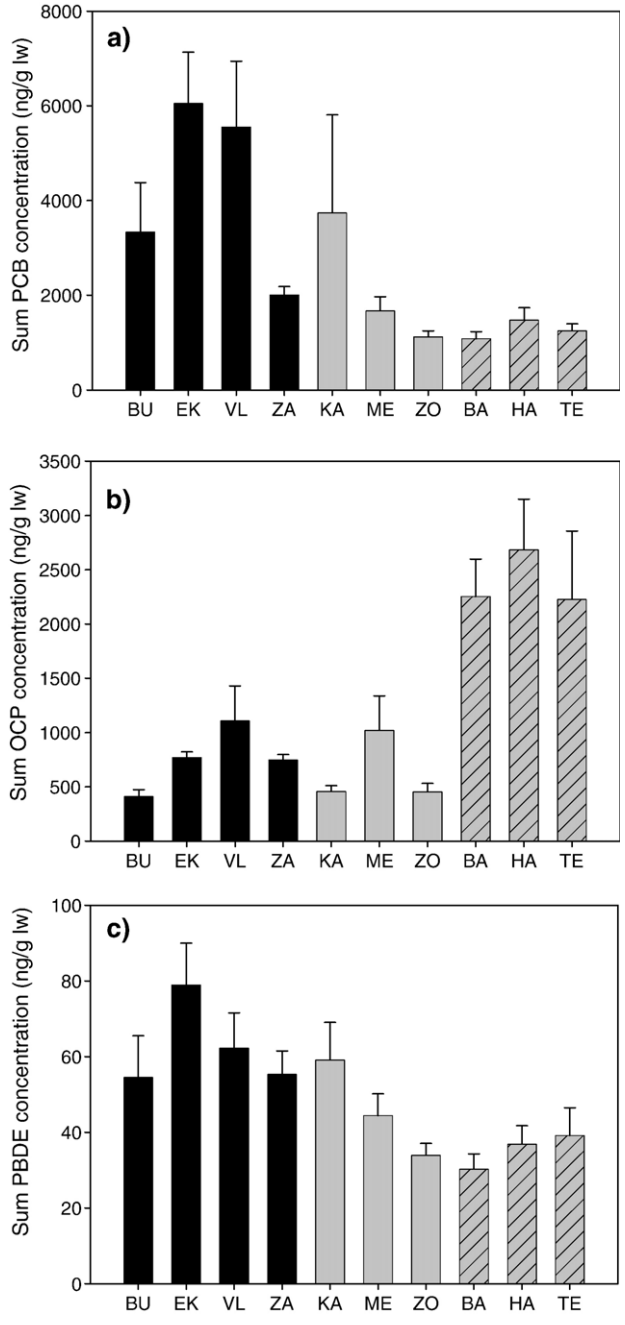

Fig. 2. Mean concentrations (with standard errors) of sum PCB (a), OCP (b), PBDE (c) concentrations in eggs of great tits from different sampling locations in Flanders ($5 \leq n \leq 7$ per sampling location); Bu: Burchtse weel, Ek: Ekeren; Vl: Vlietbos, Za: Zandvliet, Me: Meeuwen–Gruitrode, Zo: Zoersel, Ba: Baal, Ha: Ham, Te: Tessenderlo, Ka: Kalmthout, Black, grey and striped bars represent sampling locations from group I, R1 and R2, respectively.

Among the sampling locations there was a wide range in sum OCP concentrations from 411±62 ng/g lw in Burchtse Weel (Group I) to 2683±466 ng/g lw in Ham (Group R2; Fig. 2b). *p,p*'-DDE was the most abundant OCP and accounted for 91±3% of the sum OCPs. As expected, the more rural sampling locations had the highest levels of OCPs. The rural sampling locations situated in a residential area had significantly higher OCP levels compared to the other sampling locations (Nested ANOVA: $F_{2,54}=28.61$, $p<0.001$; Tukey HSD: Group R2>Group I: $p<0.001$, Group R2>Group R1: $p<0.001$; Fig. 2b). Although sampling locations from group R1 also have a rural character, sum OCP concentrations were considerably lower than in group R2 (Fig. 2b). This suggests that the local historical usage of OCPs by inhabitants may be an important source for contamination with OCPs. The difference in OCP concentrations between group R1 and group R2 may also be due to the different usage of land. The sampling locations from group R1 are characterised by the presence of pastures, while in group R2 land is more used in function of crop cultivation. A wide range of OCP levels was detected among the sampling locations. Concentrations of *p,p*'-DDT and *p,p*'-DDE were higher (about 6 to 80 times) in the present study compared to the study of Winter and Streit (1992). Although the sampling location in the study of Winter and Streit was not considered to be highly contaminated, our results suggest a higher background contamination of DDTs in Flanders. The observed levels and differences among the sampling sites can be explained by the historical usage of DDTs in the Flemish environment. Levels of *p,p*'-DDD were below the LOQ in almost all the analysed eggs, except the eggs from Ham (Group R2) and one egg sample from Zoersel (Group R1). Concentrations of HCB were also higher (about 2 to 30 times) than the concentrations in the study of Winter and Streit (1992). On the other hand, levels of β-HCH and γ-HCH in our study were lower compared to the latter study. This is in accordance with the low HCH concentrations previously found in great tit eggs and nestlings from Belgium (Dauwe et al., 2003; Van den Steen et al., 2006), which is probably due to the restricted usage of HCHs in Belgium (Breivik et al., 1999).

PBDE levels were lower than the levels of PCBs and OCPs. Sum PBDE concentrations ranged from 30±4 ng/g lw to 79±11 ng/g lw (Fig. 2c). Similarly as for PCBs, the lowest and highest concentrations were observed in the eggs from Baal (Group R2) and Ekeren (Group I), respectively. Sum PBDE concentrations were also significantly higher in the industrialised sampling locations compared to the rural locations (Nested ANOVA: $F_{2,54}=9.75$, $p<0.001$; Tukey HSD: Group I>Group R1: $p=0.046$, Group I>Group R2: $p<0.001$; Fig. 2c). This is probably also due to the vicinity of Antwerp harbour. Higher levels of PBDEs close to harbours have previously been reported in blue mussels, marine and freshwater sediments (Christensen and Platz, 2001; Voorspoels et al., 2004b). Up to date, few data exist on the levels of PBDEs in passerine eggs. In two previous studies with great tit eggs from several sampling sites near Antwerp, levels of PBDEs were similar to those from the industrialised sampling locations from our study (Dauwe et al., 2006, Van den Steen et al., 2006). Although Kalmthout is a rural area (Group R1), PBDE concentrations were also relatively high. This might also be due to contamination originating from Antwerp harbour: the

dominating direction of the wind (south west) might be responsible for the atmospheric transport of PBDEs from Antwerp harbour to Kalmthout. PBDEs are transported over long distances and are introduced into terrestrial and aquatic systems through wet and dry deposition (ter Schure et al., 2004). Wind direction has been suggested to be an influencing factor of air concentrations of PBDEs (Goel et al., 2006; Gouin et al., 2002). Although PCBs are also transported in the atmosphere, PCB concentrations in Kalmthout were medium and lower compared to the sampling locations from group I. A possible explanation for the differences between PCB en PBDE levels in Kalmthout are the higher washout ratios from the air for PBDEs compared to PCBs (ter Schure et al., 2004). PCBs are mainly found in the gaseous phase, while most of the PBDEs are detected on particles (Poster and Baker, 1996; ter Schure et al., 2004). Since particle scavenging is the dominant process for washout, PBDEs are more effectively removed from the atmosphere by precipitation than PCBs.

A significant positive correlation was found between the sum PCB and sum PBDE levels for all groups (Pearson correlation: Group I: $R=0.80$, $p<0.001$, $n=28$; Group R1: $R=0.78$, $p<0.001$, $n=18$; Group R2: $R=0.48$, $p=0.04$, $n=18$). This suggests similar exposure pathways and/or mechanisms of accumulation for these two groups of contaminants. However, maternal transfer is a highly selective process and influenced by both biological and physico-chemical factors. A biological factor which may be important for the maternal transfer of contaminants is the reproductive strategy of a bird species (Drouillard and Norstrom, 2001). On the other hand, physico-chemical properties of the contaminants, such as molecular structure and degree of halogenation, may also influence the excretion of chemicals into the egg (Bargar et al., 2001). Other studies have reported similar correlations between PCBs and PBDEs in different environmental samples (Manchester-Neesvig et al., 2001; Stapleton and Baker, 2003). There were no significant correlations between the sum PCB and sum OCP levels (Pearson correlation: $R<0.46$, $p>0.06$, for all cases) and between the sum PBDE and sum OCP levels (Pearson correlation: $-0.09<R<0.31$, $p>0.21$, for all cases). There was no effect of the sum PCB, sum OCPs and sum PBDE concentrations on egg weight, sizes and volume (Nested ANOVA: $F<1.25$, $p>0.3$, for all cases). Variance among the sampling locations was highest for the PCBs, followed by the OCPs and PBDEs (F-test: var (PCBs)>var(OCPs): $p=0.03$; var(PCBs)>var(PBDEs): $p<0.001$; var (OCPs)>var(PBDEs): $p<0.001$). The higher variation among sampling locations in PCB and OCP levels suggests that local sources are more important for contamination with PCBs and OCPs. On the other hand, the low variation in the PBDE levels suggests a minor contribution of local contamination sources for PBDEs in the sampled locations. Therefore, PBDE concentrations in our study probably reflect background contamination with PBDEs in Flanders.

3.2. Profiles of PCBs, OCPs and PBDEs

Investigating contamination profiles allows gathering important information concerning local contamination sources. Moreover, health effects are to a large extent dependent on the contaminants structure. Therefore, local contaminant profiles are important and should be taken into account for risk assessment.

CB 153, CB 180 and CB 138 were the most abundant PCB congeners and accounted together for about 70% of the sum PCBs. PCA revealed two principal components (PC1 and PC2) which accounted for 49% and 21% of the variability among the selected PCB congeners, respectively (Fig. 3a). There was a significant difference in PC2 between group I and group R1 (One-way ANOVA: $p=0.01$; Tukey HSD: $p=0.02$). The higher chlorinated, CB 180 and CB 187, contributed more to the PCB profiles of the eggs from Ekeren and Zandvliet (Group I) and from Ham

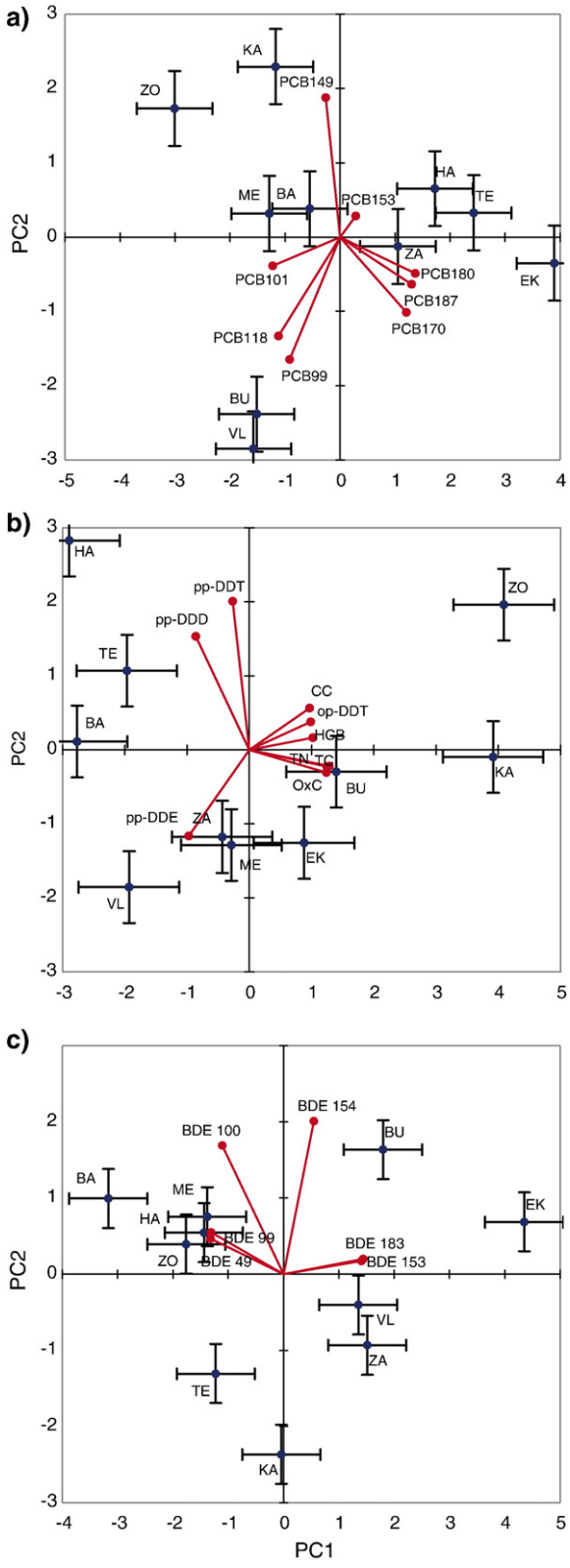

Fig. 3. Factor loadings and factor scores (±standard error) from the Principal Component Analysis for PCBs (a), OCPs (b) and PBDEs (c) in great tit eggs from different sampling locations in Flanders; Group I: Bu: Burchtse Weel, Ek: Ekeren, Vl: Vlietbos, Za: Zandvliet; Group R1: Ka: Kalmthout, Me: Meeuwen–Gruitrode, Zo: Zoersel; Group R2: Ba: Baal, Ha: Ham, Te: Tessenderlo.

and Tessenderlo (Group R2) compared to the other sampling locations (Fig. 3a). This suggests differences in PCB exposure among the sampling locations (Ormerod et al., 2000). In general, the persistence of the PCB congeners increases with the degree of chlorination. The PCB profiles of Burchtse Weel and Vlietbos (Group I) were similar, with a higher contribution of the mono-ortho substituted CB 118. Burchtse Weel and Vlietbos both have an suburban character and they are situated closely to each other, resulting in similar contamination sources. Depending on the concentrations, dioxin-like PCB congeners, like CB 118, can be responsible for adverse health effects in humans and wildlife (Carpenter, 2006). The congener profiles in Kalmthout and Zoersel, both from group R1, differed from the other sampling locations, with a higher contribution of CB 149 (Fig. 3a).

p,p'-DDE was the most abundant OCP and accounted for 91% of the sum OCPs, followed by HCB (3% of the sum OCPs) and *p,p*'-DDT (3% of the sum OCPs). Since DDE is the major breakdown product of DDT, the accumulation profile of DDTs suggests a historical input rather than contribution from recent sources. The α-HCH and γ-HCH isomers were below the LOQ in most samples. There were two principal components (PC1 and PC2) which accounted for 50% and 17% of the variability in the OCP patterns, respectively (Fig. 3b). Group R1 and R2 differed significantly in PC1 (One-way ANOVA: $p<0.006$; Tukey HSD: $p=0.02$). OCP patterns in Ham, Tessenderlo and Baal (Group R2) had a higher contribution of *p,p*'-DDD compared to the other sampling locations (Fig. 3b), in which *p,p*'-DDD was below LOQ in most samples. Besides being a breakdown product of DDT, DDD has also been used as a pesticide. Because of the rural character of Baal, Ham and Tessenderlo both contamination sources of DDD could be of importance. Higher levels of OxC, TC, TN and *o,p*'-DDT were observed in Kalmthout and Zoersel (Group R1; Fig. 3b). The differences in OCP profiles among the sampling locations are probably a result of the local usage of these pesticides, which may also be related to the usage of land.

In most cases, BDE 99 was the most abundant PBDE congener followed by BDE 47. A similar profile has been observed in two previous studies with great tit eggs (Dauwe et al., 2006; Van den Steen et al., 2006). BDE 47 and BDE 99 are the major congeners in the Penta-BDE commercial mixture (WHO, 1994). Both congeners accounted for 62% of the total sum of PBDEs. PCA revealed two principal components (PC1 and PC2) which described 65% and 19% of the variability among the PBDE congener profiles (Fig. 3c). Group I differed significantly from both group R1 and group R2 for PC1 (One-way ANOVA: $p=0.03$; Tukey HSD: Group R1: $p=0.02$, Group R2: $p=0.006$). The industrialised sampling locations from group I had a higher contribution of higher brominated PBDE congeners. BDE 153 and BDE 183 contributed highly to the profiles of Burchtse Weel, Ekeren, Vlietbos and Zandvliet. Among these sampling sites, eggs from Ekeren had the highest contribution of BDE 183 to the sum PBDEs (Fig. 3c). BDE 183 is the major congener in the Octa-BDE commercial mixture. Although the Penta-and Octa-BDE mixtures have been withdrawn from the market in Europe in 2004 (Directive EEC, 2003), the congeners present in these mixtures are still ubiquitous in the environment (Hites, 2004). It is also possible that these higher brominated PBDE congeners stem from debromination of BDE 209 coming from the Deca-BDE mixture (Van den Steen et al., 2007b), which is the only commercial BDE mixture allowed for use today (Directive EEC, 2003). In addition, BDE 154 had a higher contribution to the profiles of Burchtse Weel and Ekeren. In contrast with group I, the rural sampling locations from group R1 and R2 had a higher contribution of lower brominated congeners (Fig. 3c). Profiles in Meeuwen–Gruitrode and Zoersel (Group R1) and in Baal and Ham (Group R2) had a higher contribution of BDE 49, BDE 99 and BDE 100 (Fig. 3c). BDE 99 contributed also more to the profile of Kalmthout (Group R1) and Tessenderlo (Group R2). In general, lower brominated BDE congeners are more bioaccumulative, persistent and toxic than the higher brominated congeners (Birnbaum and Staskal, 2004; de Wit, 2002).

Our results showed less variation for the PBDE profiles compared to the profiles of the PCBs and OCPs. The first two PCs explained about 70% of the variation for the PCBs and OCPs, while 84% of the variation in PBDEs was explained. This agrees with the low variation in PBDE levels, suggesting a minor contribution from local point sources.

The present study showed that the characteristics of a sampling location influence the presence of OHPs. As expected, levels of PCBs and PBDEs were highest in the industrialised sampling locations, whereas OCPs were highest in the rural sampling locations. A higher variance among sampling locations was observed for the levels and profiles of PCBs and OCPs, compared to the PBDEs, which suggests that local point sources are more important for contamination with PCBs and OCPs than PBDEs. Differences in local contamination sources and exposure are probably responsible for the differences in contamination profiles among the sampling locations.

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